Phytochemical analysis and Gene expression profiling of *Tribulus terrestris* L. & *Pedalium murex* L.

A Synopsis of the proposed work for

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Introduction

Plants are used in modern as well as traditional medicine where they occupy a significant place for the remedy of various threatening disease (Audu *et al*., 2007). The WHO estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs for their primary health care needs. Now a day's demands for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products being non- narcotic having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor. More than 7500 medicinal plant species are reported to occur in Indian region possessing the beneficial component for various existing and anticipated diseases.

There are certain wild plants considered as weed which have a lot of medicinal values. Among them *Tribulus terrestris* (Chota Gokhuru) is important constituents of Dashmool and at the present this plants is substituted with *Pedalium murex* (Bada gokhuru) (Kevalia and Patel, 2011 and Renuka *et al.*, 2012).

Tribulus terrestris (Chota gokhuru/ Laghu Gokshur)

T. terrestris is an herb belonging to *Zygophyllaceae* family (Kustova and Dinchev, 2005). It is deciduous creeping herb native to tropical regions of the Southern Europe, Southern Asia, Africa and Northern Australia (Trease and Evans, 1996; Bhattacharjee 2004; Bayati and Al-mola, 2008; Chopra *et al*., 2009; Costa *et al*., 2010 and Moradikor *et al*., 2013). This plant is an perennial, two or more feet in length, 0.05 inch in thickness, unequal pairs of abruptly pinnate and compound leaves, small solitary stalked with perfect hermaphroditic yellowish flowers and spiny fruits at each node (Tutin *et al*., 1968 and Dymock *et al.*, 2005).

Most species having medicinal importance, but some species are weeds. This plant is tremendously rich in constituents like saponins, steroids, estradiol, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, semi drying oil, peroxides, diastase, nitrate potassium, aspartic acid, traces of glycosides and glutamic acid and other nutrients (Anita *et al*., 2013). Among these steroids, saponins are the main active component which is responsible for the diuretic activity of the plants. Dried fruits of Gokhuru are rich in protein, calcium and inorganic matters (Gauthaman and Adaikan, 2005). The plants parts are used as cooling, demulcent, diuretic, aphrodisiac, analgesic, antibacterial, anti-inflammatory, antispasmodic, anti-urolithiatic cardio-tonic, CNS depressant, emollient, appetizer, digestive, anthelmintic, expectorant, anodyne, styptic, urinary disorders and impotence. Its tonic used in increase menstrual cycle, cures gonorrhea, painful gums astringent, cough, scabies, anemia and ophthalmic (Khare 2007, Angelova *et al*., 2013, Dimitrova *et al*., 2012, Dymock *et al.*, 2005, Evan, 2005).The root is used for stomachic, appetizer, diuretic and carminative **(**Kritikar and Basu, 1975).

Pedalium murex **(Bada Gokhuru/ Brihat Gokshur)**

P. murex is a very important medicinal plant and belongs to *Pedaliaceae* family. Being non-toxic, very economical and easily available, herbal medicines have attained great preferences over synthetic drugs. It is distribute in India, Pakistan, Sri Lanka and tropical Africa. It is an herb and it is a rambling annual, much branched, spreading, succulent, glandular plant up to 50 cm tall. Leaves alternate, flower is yellow color and flowering period of this plant is from May to December and fruiting period from June to January **(**Agharkar 1991).

Pharmacologically, *P. murex* is used for anti-ulcer genic, nephroprotective, hypolepidemic, aphrodisiac, antimicrobial and insecticidal properties (Balasubramanian *et al.*, 2008; Soosairaj *et al.*, 2008; Balamurugan *et al.*, 2010 and Srinivas *et al.*, 2011). This is also used as tonic, aphrodisiac, improves appetite and useful in strangury, urinary discharges, vesicular calculi, cough, asthma, pain, cures skin diseases and heart troubles, piles and leprosy. It purifies blood, removes stone in the bladder. According to Unani system of medicine, it is used as diuretic, cures strangely, gleet, lumbago, tonic, enriches blood, increases menstrual flow, good gargles for mouth troubles and painful gums, stomachic, appetizer (Singh and Panda, 2005; Agharkar *et al*., 1991 and Das *et al*., 1966).

Despite of belonging to two different genera these plants have a similar Hindi name as Chota gokhuru/ Laghu gokshur and Bada gokhuru/ brihat gokshur (Renuka *et al.*, 2012). Both plants are tremendously rich in constituents having potential biological significance, including: saponins, steroids, estradiol, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins etc. Among these steroidal saponins is highly economic important component which is responsible for the diuretic activity of the plants (Rajashekar *et al.*, 2012). The amount of total saponins in *T. terrestris* and *P. murex* has 16% and 13% respectively (Renuka *et al.*, 2012). Saponins have been reported to inhibit proliferation and induce apoptosis of tumor cells thus reducing their invasive activity. Triterpene saponins have multidirectional effects on cancer cells (Warrier *et al*.,1996). Many pharmaceutical arrangements and food complements based on the saponin fraction from this plant are on sale worldwide (Paulina *et al*., 2015).

Although *T. terrestris* and *P. murex* can be differentiated on the basis of their morphological characteristic, yet they possess similar medicinal properties, thereby retain

moreover similar genetic profile (Jayanthy *et al.,* 2013). By having an insight of searching the genetic relativity between these plants many literatures were surveyed for this study, but no significant report was found. Most of the works are associated with morphological, anatomical, histological and little works done on phytochemical analysis of both plant. There is no report on its genomics and transcriptomics.

Differential gene expression profile is a powerful tool to identify changes in cell or tissue transcriptomes, which allows to understanding complex biological process. With appropriate controls for data quality, transcriptome analysis can be carried out to identify gene families (Lakshmi and Sabu *et al.,* 2015). Transcriptomics datasets from the medicinal plants would enable discovery of new biosynthetic genes involved in the production of medicinally important secondary metabolites across these and other taxa. The capability of next-generation sequencing to generate a near-complete transcriptome now opens the door for elucidating some of the most chemically prolific, genetically intractable, species of plants.

The aim of this study will be to study the important active ingredients between both plants and identify the potential transcripts and provide a framework of identified transcripts involved in saponin production through transcriptome assembly. The identification of transcripts study will establish a foundation for future studies related to carrying out the metabolic engineering for increasing the saponin biosynthesis and its regulation for human health benefits.

Objectives:

All parts of *Tribulus terrestris* and *Pedalium murex* are extensively collected for pharmaceutical uses. This is why both the plants have become threatened in the most part of India. In view of this the present work has been taken in the following objectives:

1. Phytochemical analysis of various parts of *T. terrestris* and *P. murex*

2. Transcriptomics of *T. terrestris.*

3. Transcriptomics of *P. murex.*

4. Comparison of gene expression pattern between two plants.

Review of literature:

Secondary metabolites have most significance role in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of these plants in ethno medicine in India (Dhandapani and Sabna, 2008). Lipid soluble compound are saponins and aglycones consisting of either a sterol or more commonly a triterpenoid and water soluble sugar residues, due to their hydrophilic nature, they are highly surface active and their biological activities are related to their chemical structures. Isolation and characterization of the saponins were carried out using NMR, HPLC, GC and TLC (Moghimipour and Handali *et al.*, 2015).

It was reported that *P. murex* is a good source of bioactive compound and traditionally use for digestive tonic, ulcers, fevers, wounds (Sermakkani and Thangapandian *et al.*, 2010). The comparative phytochemical and pharmacognostic of whole plant in *T. terrestris* and *P. murex* were carried out by Renuka *et al*., 2012. Jayanthy *et al*., 2013 demonstrated that pharmacological activity in both plant fruit and justified the use of the *P. murex* fruit as a substitute of *T. terrestris*. Same authors also reported morphologically, anatomically, histologically and phytochemically similarity between both plants. Patel *et al.*, 2012 stated that *P. murex* fruits were used for aphrodisiac against to promote the fertility rate. Patel *et al.*, 2016 stated that saponin is active component of *T. terrestris* and *P. murex* and saponin were shown high cytotoxicity against human breast cancer MCF-7 in cytotoxicity assays. Garai, 2014 reported that maximum saponin formation in shoot culture was observed in *Panax ginseng* when the medium contained 5 mg/1 IAA. The saponin contents were different in the cell culture and the cultivated plants.

Xu *et al*., 2013 reported the de novo assembly of *Setaria viridis* genome and identified the transcripts and SSR that could be used for both reverse and forward genetic studies. Zheng *et al*., 2014 described those triterpenoid saponins of *Ilex asprella*, a putative biosynthetic pathway downstream of 2, 3-oxidosqualene and candidate uni genes in the transcriptomes data that were potentially involved in the pathway. Subramaniyam *et al*., 2011 analyzed that transcriptional level of the *Gynostemma pentaphyllum* genome to identify secondary metabolite genes. The complete transcriptomes for the roots and leaves were obtained using a GS-FLX pyro-sequencer.

Gupta *et al*., 2015 first reported the functional analysis of with anolide-specific genes to elucidate chemo type as well as tissue specific with anolide biosynthesis. This genomic resource would also help in development of new tools for functional genomics and breeding in *Withania.* Reddy *et al*., 2015 performed the next generation sequencing, transcriptome analysis and predicted biosynthetic pathway of sennosides from Senna (*Cassia angustifolia* Vahl.). Huang *et al*., 2016 described comparative performance of transcriptome assembly methods for non-model organisms. Jeena *et al.,* 2017 studied the comparative transcriptome analysis of *Bacopa monnieri* and generated information about the identification, expression and differential tissues specific distribution of transcripts related to triterpenoid sapogenin.

Material and Methods:

1. **Phytochemical analysis in various parts of these plants**

Estimation of saponin in Leaf, Root and Fruit: Plant material will be homogenized with 80% methanol in 8°C, sonicated for 30 min, and centrifuged at 14,000 rpm followed by filtration. Chromatography will be performed using protocol (**Ashwani kumar, 2012)**.

2. **Isolation of m-RNA from various parts of the *Tribulus terrestris* and *Pedalium murex,* library preparation and sequencing**

a). Isolation of Total RNA: RNA will be isolated with commercially available kit.

b).Quantification of Total RNA: RNA will be quantified using Spectrophotometer.

3. **Library preparation:** The paired-end cDNA sequencing libraries will be prepared using RNA Sample Preparation kit.
4. **Quantity and quality check:** Prepared Library will be quantified on bio-analyzer.
5. **De novo sequencing:** The next generation sequencing for both plant samples of total RNA would be performed using paired-end (PE) 2x150 bp library. High quality data of plant samples will be assembled separately using RNA-Sequence assembler on optimized parameters.
6. **Comparative expression analysis:** The data generated from de novo sequencing of each plant will be compared to check common pathways and CDS and further analysis will be done using various bio-informatics tools.

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